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ANTHRAX

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The etiologic agent of anthrax, a disease primarily associated with herbivores, is Bacillus anthracis. Anthrax, the Greek word for coal, describes the black, coal-like appearance of the eschar formed during the cutaneous form of the disease seen in humans and certain other species. B. anthracis is a large Gram-positive endospore forming bacterium which exists either as spores (0.5 X 0.75 μ) or vegetative

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cells (1.0 X 3 to 6 μ). Spores are typically found only in environmental samples or body tissues exposed to atmospheric oxygen and are resistant to heat, freezing, drying, and most disinfectants. They also remain viable for long periods in animal by-products (i.e., bone meal, hides), contaminated soil, and equipment used to dispose of carcasses (17).

Upon culture on blood agar or other common laboratory media, the organism grows as long chains of rod shaped cells, likened in microscopic appearance to that of bamboo. When grown for 18 hours at 37°C under ambient atmospheric conditions on solid media, colonies are rough with slightly serrated edges measuring 3-5 mm in diameter. On 5% sheep blood agar, colonies may produce the classic medusa-head appearance around their periphery, with hemolysis being either weak or absent. When grown under 5 to 10% CO_2 on media containing 0.5% sodium bicarbonate or serum, virulent strains produce a poly-D-glutamate capsule which results in mucoid colonies of the organism.

As discussed below, B.anthracis also produces a tri-partite protein toxin in addition to the capsule; both of which are required for virulence. Genes coding for synthesis of the toxin proteins and synthesis of the capsule are on two plasmids, respectively designated pXO1, initially designated pBA1 (12, 19), and pXO2 (C. B. Thorne, personal communication). Loss of either or both plasmids gives rise to avirulent strains which are incapable of producing either or both virulence factors. Strains which have lost pXO2 are consequently toxigenic and nonencapsulated, are immunogenic, and have been used extensively as live attenuated spore vaccines in livestock (i.e., Sterne strain). Loss of plasmids during growth of B. anthracis at elevated temperatures has provided an explanation for Pasteur's success in generating attenuated vaccine strains.

INTRODUCTION

The etiologic agent of anthrax, a disease primarily associated with herbivores, is Bacillus anthracis. Anthrax, the Greek word for coal, describes the black, coal-like appearance of the eschar formed during the cutaneous form of the disease seen in humans and certain other species. B. anthracis is a large Gram-positive endospore forming bacterium which exists either as spores (0.5 X 0.75 μ) or vegetative cells (1.0 X 3 to 6 μ). Spores are typically found only in environmental samples or body tissues exposed to atmospheric oxygen and are resistant to heat, freezing, drying, and most disinfectants. They also remain viable for long periods in animal by-products (i.e., bone meal, hides), contaminated soil, and equipment used to dispose of carcasses. This report discusses cont'd

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Alkaline calcareous soils, subject to periodic flooding and formation of small pools which contain decaying plant matter, provide suitable conditions for growth of the organism and its maintenance in the environment (17). During dry weather when food becomes more scarce, animals will graze closer to the ground, thereby increasing the risk of infection from spores located there. Vectors, such as biting flies, have been shown to spread disease among goats and horses, whereas cattle and sheep typically acquire infection only through ingestion of spores.

→THE DISEASE; cont'd pg. -5-

Anthrax has long been recognized as a disease of animals and humans. Descriptions of a disease consistent with anthrax were recorded in early Hebrew, Greek, Hindu, and Roman records (7) and the disease appears to have been one of the seven Biblical plagues described in chapters 7 - 9 of Exodus. Anthrax was widespread throughout southern Europe during the 17th century. Its spread into the Western Hemisphere has been attributed to early

explorers from the Old World and settlers in the Rio Grande Valley and Mississippi delta. Introduction into Louisiana has been traced to early French settlements in the mid 19th century which resulted in widespread disease in humans and livestock. By the end of the century its incidence had spread westward into California and east into New England. Within the United States, the U. S. Department of Agriculture recognizes southeastern South Dakota, northeastern Nebraska, the Texas Gulf coast, the Mississippi River delta region, and sections of the Sacramento and San Joaquin valleys of California as anthrax districts (14). Although anthrax is well controlled in the USA, scattered, isolated cases continue to be reported in these and other parts of the country. In heavily contaminated regions of the world, that are not subject to stringent public and agriculture health laws, and where vaccination of livestock is not routinely practiced, the disease continues to cause significant economic losses. Areas which have been subject to repeated outbreaks include parts of Africa, Asia, southern Europe, Australia, and North and South America. A 1978 - 1980 outbreak in Zimbabwe, in which both animals and humans died, was attributed to poor control of cattle during the country's internal war (3).

Among domestic animals, cattle and sheep appear most susceptible to the disease, with horses and goats slightly less so. Humans are intermediate in their susceptibility to infection, whereas swine and strict carnivores are relatively resistant, especially carnivorous birds. Horses, goats, and humans may become infected through bites of flies and other insects which carry the organism subsequent to feeding on infected animals and carcasses (17).

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PATHOGENESIS

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Anthrax toxin is composed of three proteins, edema factor (EF or Factor I), protective antigen (PA or Factor II), and lethal factor (LF or Factor III), which range in molecular weight from about 83 to 89 kilodaltons (9). Both EF and LF require PA for toxic activity in that the latter may be required for entry of the other two components into host cells. Competition experiments with purified toxin proteins suggest that EF and LF mutually inhibit the action of each other, the mechanism of which has not been elucidated (8, 4). In experimental animals, intradermal injection of PA plus EF produces edema which is probably associated with the adenylate cyclase activity of EF (8). The combination of PA and LF, however, produces lethality in experimental animals (i.e., Fischer 344 rat) which experience pronounced fluid loss into the lungs and other extracellular areas in response to the mixture. Studies to date indicate that this is apparently a result of increased capillary permeability. It has been postulated that death is attributable in part to respiratory distress resulting from fluid accumulation in the lungs and to circulatory embarrassment due to pressure from accumulated fluid in the mediastinum (13). Although it is generally accepted that the toxin components are inactive individually, PA alone has been reported to produce transient alterations in neurological and cardiovascular functions in monkeys and chimpanzees (18). It is presently unclear whether EF plays a role in the lethal activity of anthrax toxin. A number of reports have shown that virulence of B. anthracis is in part due to its ability to inhibit various parameters of the host's immune system, particulary phagocytosis. The role of toxin and capsule in pathogenesis of the organism has been reviewed (15, 10).

Lincoln and Fish (10) separate the disease into two forms, cutaneous and septicemic. The cutaneous form is characterized as an intensely dark, relatively painless eschar which heals readily following antibiotic therapy. This form has been reported only in humans, rabbits, swine, and horses. The septicemic form may arise from various sources including contaminated wounds, cutaneous lesions and gastrointestinal or pulmonary infections. From these portals of entry the organism passes via the lymphatic system to the bloodstream. The mononuclear phagocytic system, especially the spleen, serves as the principal defense mechanism against the circulating bacilli, with pronounced splenomegaly a characteristic necropsy finding. Rapidly dividing organisms quickly overwhelm the mononuclear phagocytic system however, and multiple secondary sites of infection are established. In susceptible hosts a massive bacteremia ensues, with bacilli found throughout most of the body tissues. The amount of circulating toxin has been reported to parallel the extent of the bacteremia. Although there has been considerable debate concerning the actual cause of death, the toxin undoubtedly plays a paramount role in the virulence of the anthrax bacillus (10).

Cattle and sheep contract anthrax primarily through ingestion of contaminated foodstuffs. In cattle, sheep, and goats the incubation period is normally 3 to 7 days, but may range from 1 to 14 days (5). Signs of disease are variable and are often overlooked in cases of short duration, with death being the initial indication that the disease is present in a herd. Approximately 36 to 48 hours prior to death, animals may enter an acute form of the disease, characterized by severe depression and listlessness (sometimes preceded by a short period of excitement), high temperature ($42^{o}C$), dyspnea, increased heart rate, and congested hemorrhagic mucosae (1). Other symptoms

may include ruminal stasis, anorexia, abortion in pregnant cows, discolored or blood-stained milk, diarrhea, and dysentery. Local edema of the tongue and subcutaneous edema of the ventral portion of the neck and brisket, shoulder, and abdomen are evident (14). The peracute form of the disease, 1 to 2 hours duration, is characterized by sudden death of seemingly healthy animals. Typically, there is sudden staggering, collapse, a few convulsive movements, and death, which may in part result from the gross cerebral hemorrhage observed on necropsy. After death, bloody discharges from the mouth, anus, and vulva are common. Carcasses of anthrax-stricken animals are characterized by delayed or incomplete rigor mortis and blood clotting, dark (unoxygenated) blood, extremely rapid decomposition, and bloating.

Swine most frequently contract anthrax from ingestion of contaminated foodstuffs (i.e., bone meal, infected meat) and foraging on contaminated soil. The disease may be either acute or subacute, with fever, dullness, anorexia, and inflammatory edema of the face, throat, and neck (1); however, some individuals may die asymptomatically. Swelling of the throat can produce suffocation, whereas the external edematous areas are hot and usually not painful. With pharyngeal involvement, blood-stained froth may be discharged from the mouth. Petechial hemorrhages often occur on the skin. With alimentary involvement, there is dysentery, frequently without concomitant edema of the throat. Pulmonary anthrax, characterized by lobar pneumonia and exudative pleurisy, may develop following inhalation of spores (1). The acute form of the disease usually lasts 12 to 36 hours, although individual animals sometimes survive several days prior to death. Occasionally the majority of

animals within a herd become overtly ill for several days, with or without moderate swellings of the throat, followed by either a chronic form of the disease or gradual recovery (14).

In horses anthrax is almost always acute, varying in its clinical presentation according to the mode of infection. Subsequent to ingestion of spores, septicemia with enteritis and colic develop. However, when infected via insect bites, the disease is characterized by rapidly progressing subcutaneous edema of areas on the neck, thorax, abdomen, and mammary glands, which are hot and painful. Other symptoms include high fever, severe depression, anorexia, and dyspnea which occur over the course of 2 to 4 days. Anthrax in horses may be confused with colic, septicemia, acute swamp fever, and hemorrhagic purpura (14). Susceptibility of other animal species to anthrax varies.

Anthrax in carnivores usually results from eating the infected meat of carcasses. In dogs, pharyngeal or oral anthrax produces swelling about the head and throat. Alimentary involvement is typically manifested as severe gastroenteritis. Older canines are less suseptible. Poultry are highly resistant to anthrax, a characteristic which has been attributed to their higher body temperature. Other types of birds, reptiles, and fish are not susceptible under natural conditions (14). For infection to occur, the body temperature of cold-blooded animals must be raised and that of birds lowered (16).

In humans, three manifestations of the disease are recognized (listed in decreasing order of frequency): cutaneous - acquired through handling of contaminated animal products or carcasses and insect bites; gastro-

intestinal/pharyngeal - resulting from ingestion of contaminated food; and pulmonary - contracted from inhalation of spores. This last form of the disease, sometimes termed Woolsorter's disease, is virtually always fatal. As described above, all three clinical presentations may lead to septicemia and/or meningitis, the latter resulting in almost certain death.

When anthrax is suspected, necropsy should not be performed by the attending veterinarian. Since the blood of animals succumbing to anthrax clots poorly, blood or edema fluid samples may be drawn from carcasses for analysis. If necropsy has begun, both spleen and blood specimens should be submitted to state or veterinary laboratories in order to confirm anthrax as the cause of death. In cases where the carcass is decomposed, the tip of an ear is usually submitted. Exuded blood from body orifices may also be examined from recently deceased animals. Microscopic examination of stained blood smears reveals large numbers of encapsulated bacilli, either single or in short chains. Spores are not found in fresh blood samples; however, they are likely to be present in samples exposed to the atmosphere for a few hours. The capsule surrounding the bacterial cells can be visualized microscopically, in the field, in smears stained with Wrights, methylene blue, or giemsa stains. The amount of capsule detectable around the bacterial cells diminishes with the age of the specimen. Techniques, including fluorescent antibody capsule staining, lectin agglutination, glycosidase activity, toxin detection, etc. have been described for B. anthracis identification (6).

Anthrax DISEASE CONTROL

As stated above, animals infected with anthrax often do not exhibit overt symptoms until a few hours before death, at which time treatment is of little consequence. Therefore, in suspected outbreaks, prophylactic measures should be initiated for all animals as soon as possible. B. anthracis is highly susceptible to a wide spectrum of antibiotics. Penicillin and tetracycline, or their derivatives, have been reported as being most consistently therapeutically effective (5). Antibiotics should not be administered to healthy animals which have been vaccinated, since most commercial veterinary vaccines are composed of viable Sterne strain spores which must be allowed to germinate and grow in the vaccinee's body. Commercial vaccines afford protection in 7 to 10 days which lasts for about a year; thus animals require boosters on an annual basis. Animals should be vaccinated several weeks before the season when outbreaks are expected, but not within 60 days prior to slaughter for food consumption. Localized subcutaneous edema often occurs within 24 hours at the site of injection, but is rarely severe (5). Protection has historically been attributed to toxin neutralization by antibody to the toxin components. However, the possible immunological role of antibody to other antigens cannot be ruled out. This is supported by studies which demonstrate that the alum-adsorbed PA-based human vaccine is not protective against all B. anthracis strains in guinea pigs (S. F. Little and G. B. Knudson, personal communication).

Infected herds should be dealt with as soon as possible. The affected farm must be quarantined to prevent movement of animals to market or elsewhere (14). Animals at risk must be isolated until cases cease and for two weeks

thereafter. Dead animals, bedding, and soil contaminated by discharges should be disposed of through either deep burial in quick lime or by incineration. Tools and equipment should be decontaminated with 0.5% sodium hypochlorite (i.e., 1:10 dilution of laundry bleach). Exspor (Alcide Co.) has also been demonstrated to be very effective in killing anthrax spores (unpublished data, author) and is considerably less corrosive and caustic than sodium hypochlorite solutions.

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